

It is another object of the present invention to provide a method of purifying recombinantly produced uricase proteins, such as those described herein.

It is a further object of the present invention to provide a method of reducing the amount of uric acid in a body fluid of a mammal by administering a composition

containing a uricase protein of the present invention to the mammal.

It is yet another object of the present invention to provide antibodies to the uricase proteins described herein.

It is another object of the present invention to provide vectors and host cells containing the nucleic acid sequences described herein and methods of using same to produce the uricase proteins coded by same.

The present invention provides uricase proteins which may be used to produce a substantially non-immunogenic PEG-uricase that retains all or nearly all of the uricolytic activity of the unmodified enzyme. Uricolytic activity is expressed herein in International Units (IU) per mg protein wherein an IU of uricase activity is defined as the amount of enzyme which consumes one micromole of uric acid per minute.

The present invention provides a recombinant uricase protein of a mammalian species which has been modified to insert one or more lysine residues. Recombinant protein, as used herein, refers to any artificially produced protein and is distinguished from naturally produced proteins (i.e., that are produced in tissues of an animal that possesses only the natural gene for the specific protein of interest). Protein includes peptides and amino acid sequences. The recombinant uricase protein of the present invention may be a chimera or hybrid of two or more mammalian proteins, peptides or amino acid sequences. In one embodiment, the present invention can be used to prepare a recombinant uricase protein of a mammalian species, which protein has been modified to increase the number of lysines to the point where, after PEGylation of the recombinant uricase protein, the PEGylated uricase product is substantially as enzymatically active as the unmodified uricase and the PEGylated uricase product is not unacceptably immunogenic. Truncated forms of the uricases of the present invention are also contemplated wherein amino and/or carboxy terminal ends of the uricase may not be present. Preferably, the uricase is not truncated to the extent that lysines are removed.

One of ordinary skill will appreciate that the conjugated uricase-carrier complex must not contain so many linkages as to substantially reduce the enzymatic activity of the uricase or too few linkages so as to remain unacceptably immunogenic. Preferably, the conjugate will retain at least about 70% to about 90% of the uricolytic activity of the unmodified uricase protein while being more stable, such that it retains its enzymatic activity on storage, in mammalian plasma and/or serum at physiological temperature, as compared to the unmodified uricase protein. Retention of at least about 80% to about 85% of the uricolytic activity would be acceptable. Moreover, in a preferred embodiment, the conjugate provides a substantially reduced immunogenicity and/or immunoreactivity than the unmodified uricase protein. In one embodiment, the present invention provides a uricase protein described herein which can be modified by attachment to a non-toxic, non-immunogenic, pharmaceutically acceptable carrier, such as PEG, by covalent linkage to at least 1 of the lysines contained in the uricase protein. Alternatively, the uricase protein is modified by covalent attachment to a carrier through less than about 10 lysines of its amino acid sequence. Attachment to any of 2, 3, 4, 5, 6, 7, 8, or 9 of the lysines are contemplated as alternative embodiments.

The uricase protein of the present invention is a recombinant molecule which includes segments of porcine and baboon liver uricase proteins. A modified baboon sequence is also provided. In one embodiment, the present invention provides a chimeric pig-baboon uricase (PBC uricase (SEQ ID NO:2)) which includes amino acids (aa) 1-225 of porcine uricase (SEQ ID NO:7) and aa 226-304 of baboon uricase (SEQ ID NO:6) (see also sequence in Figure 5). In another embodiment, the present invention provides a chimeric pig-baboon uricase (PKS uricase) which includes aa 1-288 of porcine uricase and aa 289-304 of baboon uricase (SEQ ID NO: 4). Truncated derivatives of PBC and PKS are also contemplated. Preferred truncated forms are PBC and PKS proteins truncated to delete either the 6 amino terminal amino acids or the 3 carboxy terminal amino acids, or both. Representative sequences are given in SEQ ID NO:s 8 (PBC amino truncated), 9 (PBC carboxy truncated), 10 (PKS amino truncated) and 11 (PKS carboxy truncated). Each of the PBC uricase, PKS uricase and their truncated forms have one to four more lysines than are found in other mammalian uricases that have been cloned.

The present invention provides nucleic acid (DNA and RNA) molecules (sequences), including isolated, purified and/or cloned forms of the nucleic acid molecules, which code for the uricase proteins and truncated proteins described herein. Preferred embodiments are shown in SEQ ID NO:1 (PBC uricase) and SEQ ID NO:3

5 (PKS uricase).

Vectors (expression and cloning) including these nucleic acid molecules are also provided by the present invention.

Moreover, the present invention provides host cells containing these vectors.

Antibodies which specifically bind to the uricase proteins of the present  
10 invention are also provided. Antibodies to the amino portion to the pig uricase and antibodies to the carboxy portion of baboon uricase, when used in conjunction, should be useful in detecting PBC, or other similar chimeric proteins. Preferably, the antibody to the amino portion of the chimeric uricase should not recognize the amino portion of the baboon uricase and similarly, the antibody to the carboxy portion of the chimeric  
15 uricase should not recognize the carboxy portion of the pig uricase. More preferably, antibodies are provided which specifically bind PBC or PKS but do not bind the native proteins, such as pig and/or baboon uricases.

In another embodiment, the present invention can be used to prepare a pharmaceutical composition for reducing the amount of uric acid in body fluids, such as  
20 urine and/or serum or plasma, containing at least one of the uricase proteins or uricase conjugates described herein and a pharmaceutically acceptable carrier, diluent or excipient.

The present invention also may be used in a method for reducing the amount of uric acid in body fluids of a mammal. The method includes administering to a mammal  
25 an uric acid-lowering effective amount of a composition containing a uricase protein or uricase conjugate of the present invention and a diluent, carrier or excipient, which is preferably a pharmaceutically acceptable carrier, diluent or excipient. The mammal to be treated is preferably a human.

The administering step may be, for example, injection by intravenous,  
30 intradermal, subcutaneous, intramuscular or intraperitoneal routes. The elevated uric acid levels may be in blood or urine, and may be associated with gout, tophi, renal insufficiency, organ transplantation or malignant disease.